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Influence of vacuum filtration of Viura must on the concentration of fatty acids and their utilization in fermentation

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Fatty acids such as palmitoleic, oleic, linoleic and linolenic along with sterols constitute growth or survival factors for yeast. The objective of this work was to study the influence of vacuum filtration of Viura must on fatty acid contents and their use during fermentation. The results were compared with unclarified must, the control. Filtration drastically reduced the total fatty acid concentration (81.5%) and especially unsaturated fatty acids (97.1% of linoleic, and 100% of linolenic), as well as the minor saturated acids, arachidic and behenic. In the first half of fermentation, fatty acids were excreted in the filtered must (76.8%) whereas they were consumed in the control (46.8%). In the second half of fermentation, there was greater consumption in the control sample (74.5%) than in the filtered sample (37.4%). © 1997 Published by Elsevier Science Ltd on behalf of the Canadian Institute of Food Science and Technology

Keywords: Viura must, must clarification, fatty acids, fermentation.

INTRODUCTION

In order to produce quality white wines, it is important that the must is clarified (Houtman & du Plessis, 1981, 1986). Proper clarification of must will improve wine quality (Groat & Ough, 1978) since it eliminates pesticides (Troost, 1985), undesirable microorganisms (Williams *et al.*, 1978; Splittstoesser & Mattick, 1981) and prevents H_2S formation (Singleton *et al.*, 1975). However, if clarification is excessive, it affects the fermentation rate eliminating some nutrients and part of the wild yeast population (Reed & Nagodawithana, 1988; Ough *et al.*, 1989). Elimination of suspended solids, besides strongly reducing the amount of polyphenols and proteins (Conterno & Delfini, 1994), risks impoverishing the must's nutritive elements (Ough *et al.*, 1989; Reed and Nagodawithana, 1988); some mineral elements, sterols and unsaturated fatty acids are among those removed that are essential for yeast development (Bizeau, 1963; Siebert *et al.*, 1986; Jackson, 1994).

It has been observed that musts with large amounts of suspended solids and low concentration of oxygen ferment well because of, among other causes, fatty acid availability in the medium. Clarification by centrifugation or filtration can remove more than 90% of the fatty acid content; this is particularly marked with the unsaturated fatty acids oleic, linoleic, and linolenic (Bertrand & Miele, 1984; Mesías Iglesias *et al.*, 1991). Clarification by sedimentation with addition of gelatin or silica gel causes loses of 40 to 100% of fatty acid content (Delfini *et al.*, 1992). Eliminating these compounds during clarification of must seems to be the most influential factor in the formation of acetic acid in wine (Delfini & Cervetti, 1987, 1988; Delfini *et al.*, 1992). These authors found that in clarified musts with low fatty acid and phenolic contents and with 20% dissolved oxygen, the yeast should begin its metabolic activity, synthesizing fatty acids from pyruvic acid via acetyl-CoA. During the second half of fermentation, synthesis of unsaturated fatty acids halts upon reaching anaerobic conditions accumulating acetyl-CoA in the cells that, by hydrolysis, liberate acetic acid.

Not all the fatty acids present in must have the same importance for yeast development; the unsaturated acids palmitoleic, oleic, linoleic and linolenic, along with sterols, constitute growth or survival factors for these

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microorganisms since they affect the structure and function of its membranes (Buttke *et al.*, 1982; Traverso-Rueda and Kunkee, 1982; Quain, 1988). Among these fatty acids, oleic and linoleic have a greater effect on its development (Barber & Lands, 1973); adding $C_{18:2}$ together with $C_{18:1}$ to the medium has a great influence on transport of solutes through yeast membrane as well as in its tolerance to ethanol (Thomas *et al.*, 1978; D'Amore & Stewart, 1987). Similarly, long chain saturated fatty acids are indispensable for the development of these microorganisms (Henry *et al.*, 1971; Thurston *et al.*, 1981); however, an excessive increment in the concentration of these latter acids in yeast affects the functionality of permeases associated with the membrane and it slows the passage of sugars (Thomas & Rose, 1979; Larue *et al.*, 1982).

The composition of fatty acids in yeast varies, but the most abundant have straight chains and an even number of carbon atoms. In *Saccharomyces* the major part of the fatty acids consists of palmitic ($C_{16:0}$), palmitoleic ($C_{16:1}$) and oleic ($C_{18:1}$) (Rattray, 1988). Lipids (fundamentally phospholipids and a specific quantity of neutral lipids) along with proteins are the principal components of these microorganisms' cellular membrane (Hunter & Rose, 1971). Although the principal function of the membrane's phospholipids and sterols is to be a hydrophobic barrier between two aqueous media, protein conformation of the membrane depends on the lipid structure, and phospholipids play an important part in maintaining the natural conformation of the proteins (Esfahani *et al.*, 1979; Esfahani & Devlin, 1982).

The aim of this study was to observe the influence of clarification by vacuum filtration on fatty acid content and their utilization during the fermentative process in *Vitis vinifera* var. Viura musts. Must was obtained and maceration took place in a wine cellar, and the fermentation was sited in a pilot plant to simulate industrial conditions. The must and wines obtained were analyzed and compared with control must (unclarified).

MATERIALS AND METHODS

Samples and vinification

Vitis vinifera var. Viura grapes of Navarra Denomination of Origin were crushed and destemmed to make must for subsequent production of white wine in the pilot plant. The skins were not removed for 6 hours. Must was then divided into two fractions. The first one was treated with SO_2 ($50\,mg\,l^{-1}$) but was not subjected to any prefermentation technique. The other, following refrigeration at 10 °C and addition of SO_2 ($50\,mg\,l^{-1}$), was clarified by rotary vacuum filtration. Then 400 l of each must was subjected to fermentation using $0.5\,g\,l^{-1}$ of Fermivin active dry S. *cerevisiae* from Gist-brocades (F. Lafford and Cia., Pasajes, Spain). The temperature was controlled at 17.7 °C with a standard deviation of less than 2 °C. In both cases, the fermentation was continued until the concentration of reducing sugars fell below $2.5\,g\,l^{-1}$.

A stainless crusher-stemmer, Marzola Marzinox (Marrodan and Rezola SA, Logroño, Spain) equipped with a rubber roller, was used to destem and crush the grapes. The must was filtered through a rotary vacuum filter, Espal V-20 (Temavinsa, Logroño, Spain), with a 6500 l measuring barrel, equipped with 4 hp shaking motor, a 40 hp vacuum pump and a 7.5 hp feed pump. The diatomaceous earth filter, with a maximum particle size of 52 μm, had a surface area of $30\,m^2\,g^{-1}$ and a filtration volume of 8000 to $10000\,L\,h^{-1}$. The turbidity of the must was determined using a model 18900 Hach turbidimeter (Hach Co., Loveland, CO), prepared for colored samples.

Vinification was carried out in stainless steel (AISI 316-18/8/2) vertical tanks. Tank dimensions were 0.76 m diameter and 1.1 m height, and the capacity was 400 l.

Extraction and GC analysis of fatty acids

Determination of fatty acids was performed on a Perkin-Elmer 8420 gas chromatograph (Perkin-Elmer Corporation, Norwalk, CO, USA) equipped with flame ionization detector and a fused silica capillary column (Supelcowax 10; 30 m × 0.25 mm i.d.). The chromatograph was connected by a Waters interface (Waters Chromatography Div., Milford, MA USA), and Maxima 820 software was employed for the acquisition and processing of data. The volume injected, equally for the samples and standard solutions, was 1 μl. The injector and detector temperature was 200 °C. The initial oven temperature was 120 °C, increased at 3 °C per min up to 200 °C, and was maintained for another 35 min. Helium was the carrier gas.

Standard solutions for the analysis of the fatty acids (Matreya Inc., Pleasant Gap, PA, USA) by GC were prepared for different concentrations at intervals from 10 to $250\,mg\,l^{-1}$.

Internal standards were methyl heptadecanoate and methyl undecanoate (Sigma Chemical Co., St. Louis, MO, USA).

The lipid fraction was extracted by the procedure of Darné and Madero-Tamargo (1979). Fatty acid determination was carried out following the method recommended in the Código Alimentario Español (1977), which is based on the formation of methyl esters, by transesterification of the esters present and esterification of free fatty acids, and their subsequent extraction. The lipid extraction was performed with a mixture of chloroform, ethanol and double distilled water (2:1:1). For the preparation of methyl esters, sodium methoxide 0.2 M (0.5 g of sodium metal in 100 ml of anhydrous methanol) and hydrochloric acid at 4% (w/w) in methanol (prepared by passing a stream of hydrogen chloride through anhydrous methanol) were used. Methyl esters were extracted with a mixture of hexane/

H_2O 2:10 (v/v). Reagents employed were from Panreac (Montcada i Reixac, Barcelona, Spain).

Enological parameters

The enological parameters were measured according to the methods described by the Office International de la Vigne et du Vin (1990).

All determinations were performed in quadruplicate on representative samples of musts and wines. The results given in tables are with standard errors (SE). To improve clarity, the results represented in histograms do not include SE; however, the variation coefficients for fatty acid data obtained by the method described were between 1.7–11.0.

RESULTS AND DISCUSSION

General characteristics of musts and wines

In Table 1 it is observed that rotary vacuum filtration reduced must turbidity by 86% with respect to the control since it retains macromolecules (neutral polysaccharides, proteins, pectins and polyphenols) (Villetaz *et al.*, 1981). Also, the filtered must had 11.8% less residual ash with respect to the control, probably due to the filter's elimination of organic salts or of complexes formed by proteins with iron or copper (Bayly & Berg, 1968; Gorinstein *et al.*, 1971; Gorinstein, 1975). However, this treatment did not affect initial sugar concentration nor the pH of the must, but did slightly modify the value for total acidity.

In spite of the fact that the clarified must fermented much slower than the control, both fermented to dryness with residual sugar content less than 2.5 $g l^{-1}$, and the alcoholic degree reached in both wines was similar. The volatile acidity of the wine from the filtered must was greater than that of the control, but both values were within the normal interval (0.12 to 0.30 $g l^{-1}$ acetic acid) described by Amerine and Ough (1976) and, as such, presented no problems in their conservation nor in their organoleptic characteristics.

Fatty acids of initial must

Filtration drastically reduced the total fatty acid concentration (81.5%) with respect to the control (Table 2). This clarification treatment completely eliminated the acids arachidic, behenic and linolenic (Fig. 1). Similarly, Miele *et al.* (1993) observed elevated losses of total fatty acids (93.6%) in filtered musts of Cabernet Sauvignon. Bertrand and Miele (1984) and Castelá *et al.* (1985) concluded that centrifugation or filtration of must totally eliminated some fatty acids.

The unsaturated/saturated fatty acids relation fell dramatically with filtration (from 1.2 in unclarified must to 0.2 in the filtered) (Table 2); this is due to filtration especially eliminating unsaturated fatty acids (100% of linolenic and 97.1% of linoleic). The filter retains, among other things, skins and pips of the berry where linoleic acid is stored, 24% and 62%, respectively (Mesías Iglesias *et al.*, 1991). Similarly, linoleic acid is one of the predominant fatty acids in suspended solids that are separated by centrifugation (Schisler *et al.*, 1982). In filtered must, the efficient elimination of unsaturated fatty acids coincides with a decreased rate of fermentation (Ayestarán *et al.*, 1995). The disappearance of lipids contained in the skin, through clarification of must, reduces the fermentative power of yeast (Lafon-Lafourcade, 1983).

Evolution of fatty acid concentration in the first half of fermentation.

In Fig. 2 the change in fatty acid concentration is represented for both samples during this step. In the filtered must, total fatty acid concentration increased by 76.8%, but in the control it decreased to 46.8% (Table 2). In the filtered must, the low total fatty acid concentration (6 $mg l^{-1}$) and the lack of some of them compels yeast to synthesize these acids (Delfini *et al.*, 1992); this would retard its development, as was reflected by the slower rate of fermentation in this step in contrast to the control.

The total concentration of medium chain fatty acids ($C_{8:0}$, $C_{10:0}$ and $C_{12:0}$) increased in both musts, reaching

Table 1. Evolution of general parameters of Viura musts and the wines produced during fermentation. All parameters listed with their standard error (n = 4).

		Turbidity (NTU[a])	Reducing Sugars ($g l^{-1}$)	pH	Total Acidity ($g l^{-1}$[b])	Volatile Acidity ($g l^{-1}$[c])	Ash ($g l^{-1}$)	Alcohol (v/v %)
Filtered	Must	97 ± 3	181.6 ± 0.5	3.47 ± 0.01	4.3 ± 0.1		2.9 ± 0.1	
	Mid-point of fermentation		73.4 ± 0.6	3.31 ± 0.01	4.36 ± 0.02		2.05 ± 0.04	
	Wine recently produced		0.73 ± 0.06	3.37 ± 0.1	3.44 ± 0.02	0.25 ± 0.01	1.44 ± 0.05	10.5 ± 0.1
Control	Must	695 ± 7	179.7 ± 1.7	3.51 ± 0.01	4.11 ± 0.01		3.4 ± 0.1	
	Mid-point of fermentation		74 ± 2	3.32 ± 0.01	4.52 ± 0.01		3.1 ± 0.2	
	Wine recently produced		0.45 ± 0.08	3.28 ± 0.01	4.85 ± 0.02	0.14 ± 0.01	1.6 ± 0.1	10.7 ± 0.1

[a]Nephelometric turbidity units.
[b]As $g l^{-1}$ tartaric acid
[c]As $g l^{-1}$ acetic acid

Table 3. Total fatty acid concentrations of filtered and control Viura musts and of the wines produced

Fatty acids	Initial must		Mid-point of fermentation		Wine recently produced	
	filtered	control	filtered	control	filtered	control
Medium chain[a]	1.04	2.1	6.5	5.7	4.1	1.5
Long chain saturated[b]	4.0	12.8	2.4	6.6	1.3	1.4
Long chain unsaturated[c]	0.9	17.3	1.6	4.8	1.1	1.0
Total	5.9	32.2	10.5	17.1	6.5	3.9

[a]Sum of concentrations of $C_{8:0}$, $C_{10:0}$, $C_{12:0}$
[b]Sum of concentrations of $C_{13:0}$, $C_{14:0}$ $C_{15:0}$, $C_{16:0}$,$C_{18:0}$, $C_{20:0}$, $C_{22:0}$
[c]Sum of concentrations of $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$

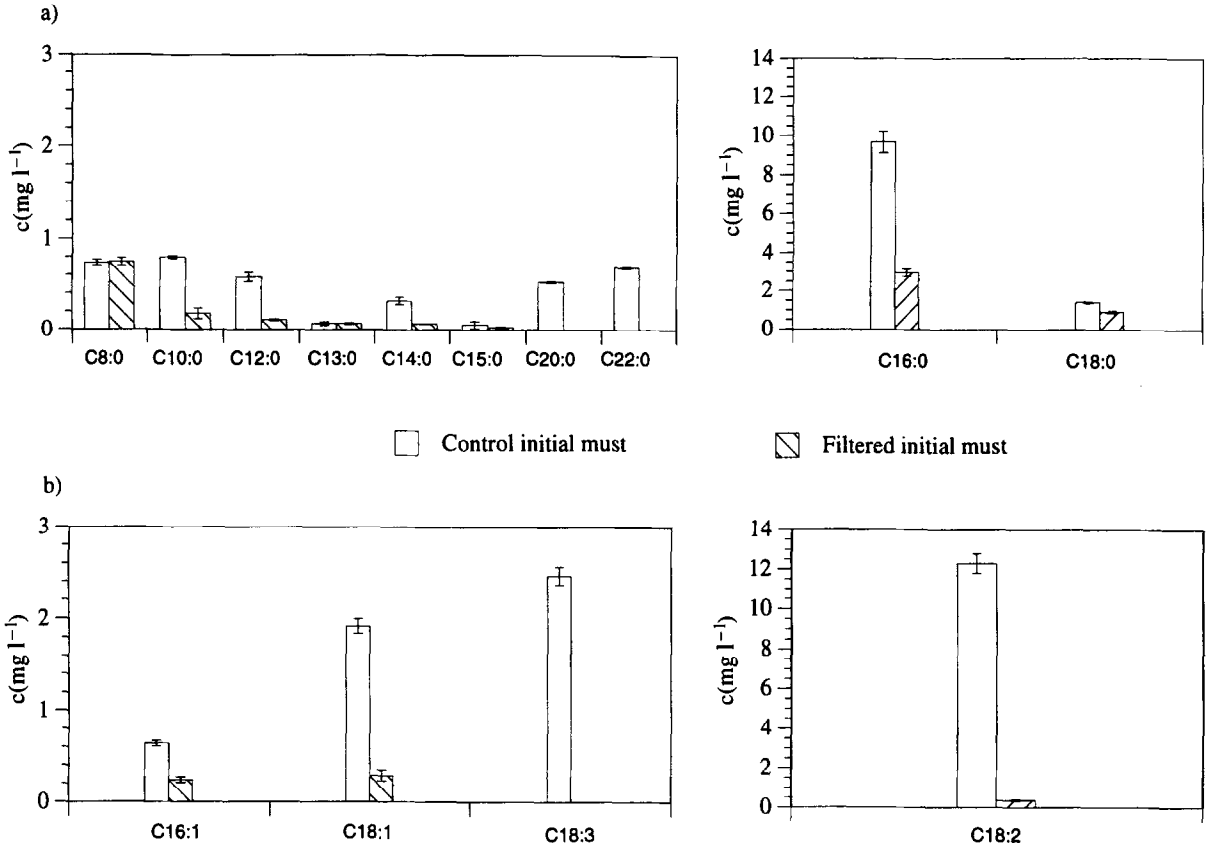

Fig. 1. Concentration of fatty acids at the beginning of fermentation: Viura must clarified by filtration and unclarified control must (n = 4). (a) Saturated fatty acids; (b) unsaturated fatty acids. All results are shown with standard deviation.

a value of 6.53 mg l^{-1} in the filtered and 5.7 mg l^{-1} in the control; this represents 62.7 and 46.6%, respectively, of total fatty acid content in this step (Table 2). Edwards *et al.* (1990) also observed that in clarified musts of the var. Aurora, there had been greater production of $C_{6:0}$, $C_{8:0}$ and $C_{10:0}$ than in unclarified musts. The medium chain fatty acid concentration depends on the balance between its production and utilization by yeast for the synthesis of long chain fatty acids (Viegas *et al.*, 1989). Probably, less turbidity in filtered must produced greater over-saturation of CO_2 (Thomas *et al.*, 1994), which caused early inhibition of unsaturated fatty acid synthesis and favored liberation of medium chain fatty acids (Aries *et al.*, 1977). At the mid-point of fermentation, the sum of $C_{8:0}$ and $C_{10:0}$ in filtered must (4.63 mg l^{-1}) and control (3.83 mg l^{-1}) was greater than the limit value (3 mg l^{-1}) at which the yeast population in a synthetic medium reduces (Geneix *et al.*, 1983). In filtered must, the low colloidal content was, possibly, not sufficient to adsorb these acids allowing their toxic action (Ollivier *et al.*, 1987); this, along with other factors, would influence the evolution of fermentation.

The total concentration of long chain fatty acids ($C_{13:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{18:0}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:0}$ and $C_{22:0}$) decreased less in the filtered must (19.3%) than in the control (62.1%) (Table 2). Consumption of these acids is due, in part, to yeast, in the first days of fermentation, being able to incorporate fatty acids and triglycerides from the medium, increasing up to four times its content of $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ and $C_{18:1}$ (Rosi & Bertuccioli, 1992).

Long chain saturated fatty acids were similarly

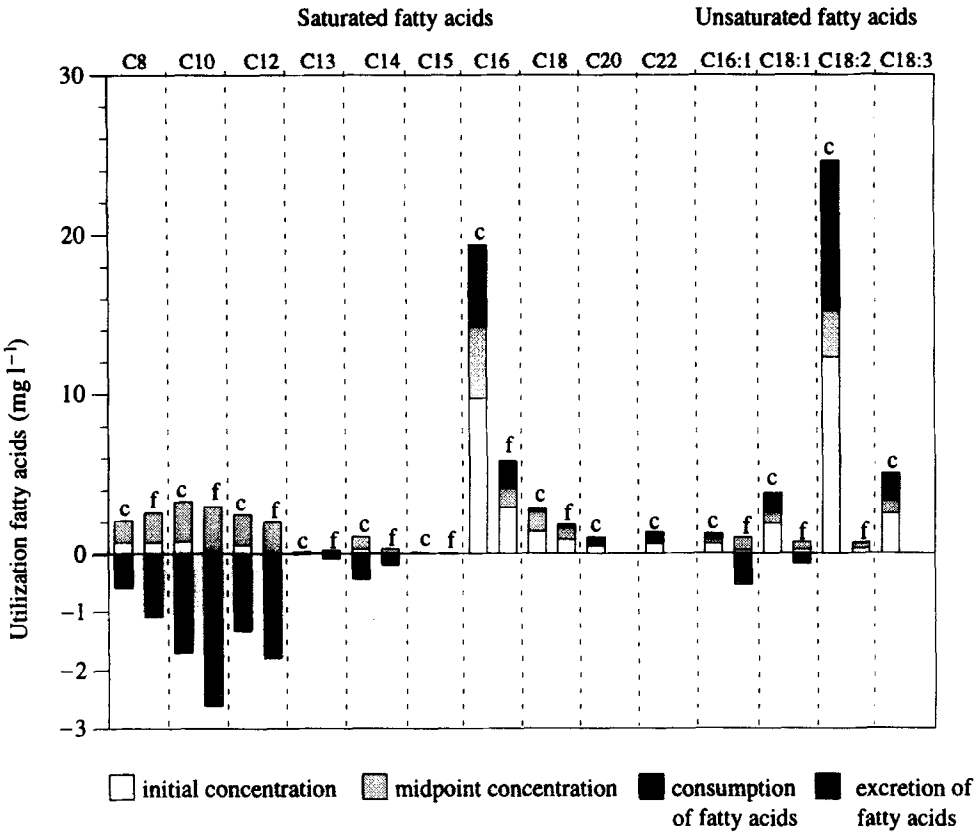

Fig. 2. Concentration of fatty acids in Viura must at beginning and mid-point of fermentation ($n=4$). Consumption or excretion of fatty acids during the first half of fermentation (c = control must sample; f = vacuum filtered must sample)

consumed, in filtered must 40.6% and in the control 48.5%. In both cases, palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids were consumed principally; they are usual components of yeast (Ratledge and Evans, 1989) and necessary for correct functioning of sugar and amino acid transport systems (Otoguro *et al.*, 1981). The amount of myristic acid ($C_{14:0}$) increased in both samples. This agrees with Herráiz *et al.* (1990) who found that yeast synthesized and excreted this fatty acid during the first phase of fermentation. On the other hand, filtration completely eliminated some very long chain acids ($C_{20:0}$ and $C_{22:0}$), whereas in the control must 100% of both acids were consumed (Fig. 2); possibly, the availability of these acids in the control must favored yeast generation and, as such, its development (Ratledge and Evans, 1989).

In the control must, 72.1% of the unsaturated fatty acids were consumed whereas, in the filtered, 76.4% were excreted (Table 2). Delfini *et al.* (1992) indicated that the presence of unsaturated acids in the medium favored the fermentative process; thus, in the control must the presence of these fatty acids will favor the development of yeast. In the clarified must, the concentration of $C_{16:1}$ and $C_{18:1}$ increased (Fig. 2); this increase might, at the beginning of this fermentation step, be due to the minor concentration of both acids which forced the yeast to activate its synthesis of them, a process favored by the presence of oxygen in the medium (Aries *et al.*, 1977). On the other hand, at the end of this step, the over-saturation reached by CO_2 in clarified media modifies the lipid content and the permeability of the membrane (Calderbank *et al.*, 1984, 1985) which, probably, gives rise to the loss of $C_{16:1}$ and $C_{18:1}$ to the medium. Polyunsaturated acids $C_{18:2}$ and $C_{18:3}$ cannot be synthesized by *S. cerevisiae* (Rosi and Bertuccioli, 1992). Acids $C_{18:2}$ along with $C_{18:1}$ are the acids that most activate the growth and development of *S. cerevisiae* (Rattray *et al.*, 1988). Thus, eliminating $C_{18:3}$ by filtration and not consuming $C_{18:2}$ will contribute to the decreased fermentation rate of filtered must with respect to the control.

Evolution of fatty acid concentration in the second half of fermentation.

A smaller percentage of total fatty acid concentration was consumed in the filtered sample (37%) than in the control (78.3%) (Table 2). In Fig. 3 the changes in fatty acid concentration are shown for this step.

The total concentration of medium chain fatty acids ($C_{8:0}$, $C_{10:0}$ and $C_{12:0}$) decreased a lesser percentage in wine produced from filtered must (37.4%), than in the control (74.5%). Similarly, Dubourdieu *et al.* (1980) found greater concentrations of $C_{6:0}$, $C_{8:0}$, $C_{10:0}$ and $C_{12:0}$ in wines made from musts that contained low quantities of solids in suspension. In the filtered sample,

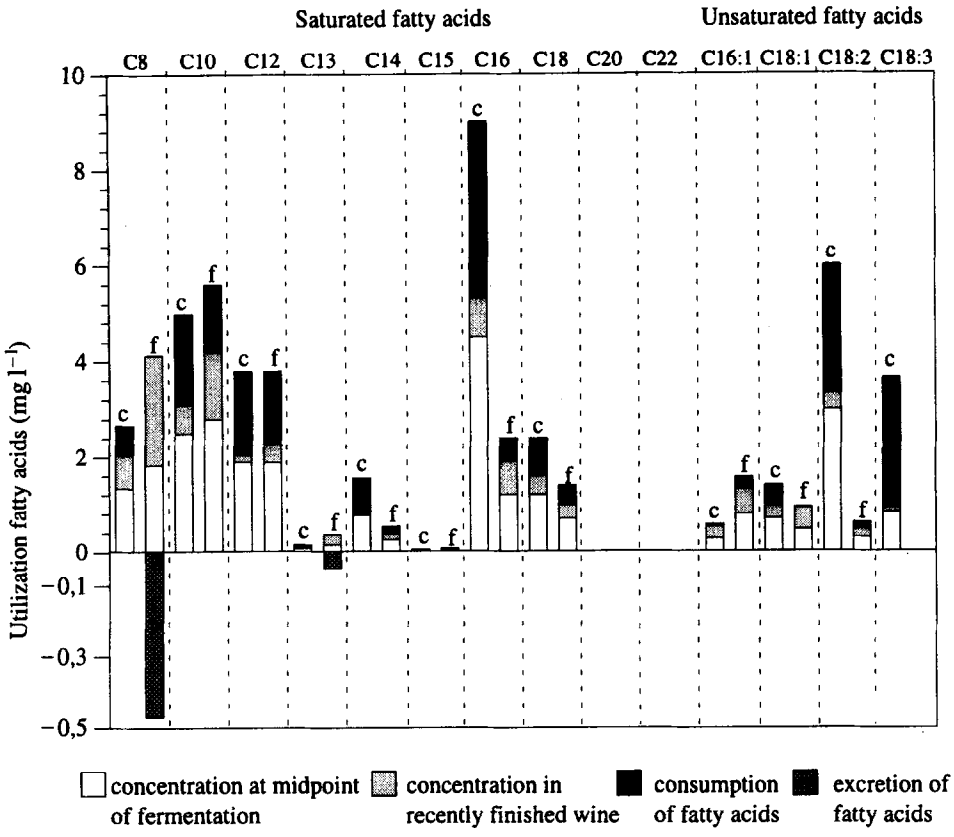

Fig. 3. Concentration of fatty acids in Viura must at mid-point of fermentation and in recently finished wine (n = 4). Consumption or excretion of fatty acids during the second half of fermentation (c = control must sample; f = vacuum filtered must sample).

$C_{10:0}$ decreased a smaller percentage (50%) than in the control (76%), and in both samples $C_{12:0}$ diminished similarly (79.5% in the filtered and 92.1% in the control). The concentration of $C_{8:0}$ increased in the filtered sample, and decreased in the control (Fig. 3). With regard to the toxicity of these fatty acids, Sá-Correia *et al.* (1989) found that $C_{10:0}$ was more toxic for yeast than $C_{8:0}$. In the literature, there is ambiguity with respect to the utilization of these acids. Herráiz *et al.* (1990) observed in musts of variety Albillo an increase of $C_{8:0}$ and $C_{10:0}$ until the end of fermentation; Ravaglia and Delfini (1993) confirmed that in synthetic medium these acids diminish starting from the seventh and eighth days of fermentation. Geneix *et al.* (1983) and Larue *et al.* (1982) verified that $C_{8:0}$ and $C_{10:0}$ can be adsorbed on the remains of yeast cell walls, $C_{10:0}$ more so than $C_{8:0}$. However, Lafon-Lafourcade *et al.* 1984 proposed the possibility that this sorption occurred also on the walls of living yeast. Possibly, both phenomena would contribute to the change in concentration of these medium chain acids at the end of the control must's fermentation.

The total concentration of saturated long chain fatty acids ($C_{13:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$ and $C_{18:0}$) decreased a smaller percentage in the filtered sample (43%), than in the control (79.2%) (Table 2). In both samples, the high consumption of $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ was noteworthy although it was smaller in the filtered sample ($C_{14:0}$, 59.3%; $C_{16:0}$, 41.7%; $C_{18:0}$, 58.6%) than in the control ($C_{14:0}$, 92.3%; $C_{16:0}$, 81.8%; $C_{18:0}$, 65.8%) (Fig. 3). Herraiz *et al.* (1990) found in variety Albillo grapes that the content of $C_{14:0}$ and $C_{16:0}$ decreased at the end of fermentation and $C_{18:0}$ slightly increased. At the end of fermentation, yeast consumes these acids when they can no longer synthesize them, since lipid synthesis halts just before its cellular division ceases (Thurston *et al.*, 1981).

In this step, total unsaturated fatty acid concentration decreased a lesser percentage in the filtered sample (26.7%) than in the control (80.1%) (Table 2). There are very notable differences in utilization of these acids between both samples. Whereas no $C_{18:3}$ existed in the filtered must, in the control it was consumed by 88%. The acid $C_{18:1}$ was not consumed in the filtered sample, but in the control it was consumed by 61.4%. A different behavior was shown for the acid $C_{16:1}$, it was slightly consumed in the control sample, but 33% was consumed in the filtered. Finally, $C_{18:2}$ was consumed similarly in both samples (48.4% in the filtered and 59% in the control) (Fig. 3).

In the clarified sample, the low consumption of unsaturated fatty acids probably implicates major saturation of yeast's plasma membrane; this will lessen its resistance to the toxic action of ethanol (Ingram *et al.*, 1986) and alter membrane function which affects the transport systems of some monosaccharides like glucose (Rattray *et al.*, 1975; Watson 1980). To this effect, Cavazza *et al.* (1985) confirmed that, in anaerobic conditions, the

immediate response of yeast in contact with ethanol was to increase the percentage of unsaturated fatty acids, in particular $C_{18:1}$; this did not occur in the filtered sample (Fig. 3).

In the control sample, similar consumption of saturated and unsaturated fatty acids in this step will permit better regulation of the saturated/unsaturated relationship in the membrane; this was reflected in the greater rate of fermentation for this sample.

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